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Tumor Necrosis Factor Alpha Promoter Polymorphism at Position -238 Is Associated With Chronic Active Hepatitis C Infection

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Tumor necrosis factor α (TNF-α) is involved in the pathogenesis of chronic hepatitis C virus infection. The gene for TNF-α is encoded in the major histocompatibility locus (MHC). Two polymorphisms at positions -308 and -238 in the TNF-α promoter region might influence TNF-α expression. These promoter polymorphisms have been linked previously to a number of infectious diseases. TNF-α promoter polymorphisms at positions -238 and -308 were studied by DNA sequencing and sequence-specific oligonucleotide hybridization in 82 individuals with chronic hepatitis C and 99 control subjects. Subjects had been HLA class I and class II typed in a previous study. The frequency of the TNF238.2 promoter allele was significantly higher in the hepatitis C group (18.7%) compared to the controls (3.5%; $P < 0.0001$; pcorr < 0.009). No significant differences in the frequency of the TNF308.2 allele were observed between patients and controls. The increased frequency of the TNF238.2 allele could not be explained by linkage disequilibrium to HLA-B or -DR genes. These findings show an association between the TNF238.2 promoter variant and chronic active hepatitis C. They suggest that this polymorphism or a linked gene may be a host factor contributing to the development of chronic active hepatitis C. ***J. Med. Virol. 54:173–177, 1998.***

KEY WORDS: major histocompatibility complex; viral clearance; cytokines; linkage disequilibrium

INTRODUCTION

Infection with the hepatitis C virus (HCV) leads to chronic disease in 50–70% of patients [Alter et al., 1992]. The outcome of acute hepatitis C infection appears to be determined by the vigor of the anti-viral T-cell immunoresponse [Diepolder et al., 1995; Missale et al., 1996]. Despite rapidly increasing knowledge about viral structure, subtypes, and the pathogenesis, little is known about host factors influencing the course of the disease. Two recent studies have reported altered gene frequencies of MHC-class II genes in patients with chronic hepatitis C. The frequency of DR13 appears to be increased in patients with little disease activity [Kuzushita et al., 1996] and decreased in patients with chronic active disease [Höhler et al., 1997]. In addition DR3 has been associated with chronic active disease [Höhler et al., 1997]. Thus, there appears to be an association between the course of hepatitis C infection and immune response genes.

Several lines of evidence suggest the importance of TNF-α in hepatitis C infection. Patients with acute and chronic hepatitis C have elevated plasma levels of TNF-α [Tilg et al., 1992; Torre et al., 1994]. Liver infiltrating cytotoxic T lymphocytes (CTL) have been shown to secrete TNF-α and interferon-γ [Löhr et al., 1994; Koziel et al., 1995]. In addition HCV infection induces TNF-α production in human hepatocytes [González-Amaro et al., 1994]. A recent investigation indicated that nonresponsiveness to interferon-α treatment is related to high pretreatment TNF-α levels [Larrea et al., 1996].

The gene for TNF-α is located within the class III region of the MHC between HLA-B and -DR. Its expression is tightly controlled at the transcriptional and posttranscriptional level. Two G vs. A transitions in the promotor region at positions -308 [Wilson et al., 1993] and -238 [D'Alfonso and Richiardi, 1994] have been shown to influence TNF-α expression. At position -308 allele 2 (A; TNF308.2) is associated with higher consti-

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TABLE I. PCR Probes and Washing Temperaturs Used for Detection of TNF-α Promoter Alleles With Dot-Blot Hybridization

Probe	Sequence	Position	Washing temperature
TNF-G308	5′CCCGTCCCCATGCCCCT3′	−301−−317	56°C
TNF-A308	5′AGGGGCATGAGGACGGGG3′	−317−−300	56°C
TNF-G238	5′CCCTGCTCCGATTCCGAG3′	−230−−247	56°C
TNF-A238	5′CCTCGGAATCAGAGCAGGG3′	−248−−230	57°C

tutive and inducible levels of TNF-α [Wilson et al., 1994] whereas for the TNF238.2 (A) allele at -238 functional consequences are not yet clear [Pociot et al., 1995]. The TNF308.2 allele has been linked with susceptibility to cerebral malaria [McGuire et al., 1994], mucocutaneous leishmaniasis [Cabrera et al., 1995], whereas both TNF308.2 and TNF238.2 have been associated with tuberculosis and malarial anaemia [Hill et al., 1996].

The aim of the study was to determine whether TNF-α promotor polymorphisms influence the disease course in hepatitis C infection.

MATERIALS AND METHODS

Study Subjects

Eighty-two Caucasian patients with chronic active hepatitis C (34 female, 48 male) were enrolled prospectively in the study. Informed consent was obtained from each patient and the study was approved by the local ethics committee. The diagnosis of chronic hepatitis C was established by the finding of antibodies to HCV by a second generation enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories, Delkenheim, Germany) and the detection of HCV-RNA by PCR (Amplicor, Hoffmann-La Roche, Grenzach-Whylen, Germany) in all patients. Patients had been infected with HCV for more than 2 years. All patients had elevated serum alanine aminotransferase levels. In addition antinuclear antibodies (ANA), smooth muscle antibodies (SMA), antimitochondrial antibodies (AMA), soluble liver antigen antibodies (SLA), and antibodies to liver/kidney microsome type 1 (anti-LKM1) were measured in all patients repeatedly to exclude autoimmune hepatitis. None of our patients was positive for LKM-1, SLA, or AMA. Antibody titers in patients positive for SMA (five patients) and ANA (one patient) never exceeded 1:40, the lowest titer considered positive in the test assays. The presence of antibody usually followed α-interferon therapy and antibodies disappeared on subsequent testing. All patients were without HBsAg and other causes of liver disease. Histological examination showed chronic hepatitis in 65 of 82 subjects (79.3%) and in 17 of 82 patients (20.7%) chronic hepatitis with transition to cirrhosis. The normal control population consisted of 99 unrelated Caucasoid persons from routine consecutive paternity cases investigated at the Institute of Legal Medicine. In a previous study 73 of the 82 subjects with chronic hepatitis C virus infection had been tested for HLA class I antigens by standard microlmphocytotoxicity assay and for DRB1 alleles by allele specific PCR and PCR-SSO (hybridization with sequence specific oligonucleotides), respectively [Höhler et al., 1997].

TNF-α Promoter Polymorphisms

A 328 bp fragment spanning position -396 to -69 of the 5′untranslated region of the TNF-α gene was amplified using primers TNF-396 (5′TTCCTGCATCCT-GTCTGGAA 3′) and TNF-69 (5′CAGCGGAAAACT-TCCTTGGT 3′). A panel of samples typed previously for HLA-DR was typed initially for the polymorphisms by sequencing the entire 328 bp fragment. This led to the identification of a number of heterozygous and homozyous individuals for each of the four polymorphisms. TNF promoter alleles in the study subjects were detected by dot blot analysis of amplified DNA incorporating in every analysis a panel of sequenced reference samples. Four digoxigenin labeled oligonucleotide probes were used for the identification of the promoter alleles (Table I). Filters were hybridized at 40°C and washed with TMAC at the temperatures indicated in Table I. Hybridization and color detection were carried out according to the manufacturers instruction (Boehringer Mannheim, Germany).

Statistical Analysis

The frequencies of TNF-α promoter alleles were compared between patients with chronic HCV infection and controls by chi square tests with Yates correction for small numbers. When multiple comparisons are made significant associations may arise by chance. To avoid such errors *P* values were corrected with the number of alleles tested in the entire associations study (18 for HLA-A, 38 for HLA-B, 10 for HLA-C, 18 for DRB, and 4 for TNF-α; 88 alleles tested). Relative risks associated with a particular allele were calculated using a χ^2 distribution: RR = (n1 × n4)/(n2 × n3), where n1 is the number of patients with allele x, n2 is the number of controls with allele x, and n3 and n4 are the corresponding proportions of individuals in patient and control groups not carrying allele × [Dyer and Warrens, 1994]. In each group the distribution of TNF-α promoter genotypes was checked for deviations from Hardy-Weinberg equilibrium using an exact test [Guo and Thompson, 1992]. Linkage disequilibrium between HLA-B, DRB1*, and TNF-α was tested by χ^2 test and by an exact test [Zaykin et al., 1995]. *P*-values were corrected for the number of alleles tested (38 for HLA-B, 18 for DRB and 4 for TNF-α; 60 alleles tested). *P*-values < 0.05 were regarded as significant.

TABLE II. Genotype Distribution for TNF-α Promoter Variants at Position −238 and −308 in Control and Chronic Hepatitis C Subjects*

	Controls (n = 99)		Chronic hepatitis C (n = 82)			
Locus/genotype	n	%	n	%	p	RR
TNF-α 238						
G/G	92	93	56	68	[a]$P < 0.0003$, $p_{corr} < 0.017$	
G/A	7	7	23	28	[b]$P < 0.0008$, $p_{corr} < 0.06$	5.1
A/A	0	0	3	4	n.s.	
TNF-α 308						
G/G	73	74	56	68	n.s.	
G/A	20	20	23	28	n.s.	
A/A	6	6	3	4	n.s.	

*n = number of investigated patients; n.s. = not significant.
[a]G/A and A/A vs. G/G in hepatitis C patients compared to controls.
[b]G/A vs. G/G in hepatitis C patients compared to controls.

RESULTS

Table II shows the genotype frequencies of the four TNF-α promoter variants. The corresponding gene frequencies are shown in Table III. The frequency of the TNF238.2 variant (A at position -238) was significantly higher in the hepatitis C group (homozygotes and heterozygotes AA and GA vs. GG; $P < 0.0003$; $P_{corr} < 0.017$). There were no TNF238.2 homozygotes in the control group, but we found three homozygous HCV infected subjects. Correspondingly the gene frequency of the TNF238.2 allele was significantly elevated compared to the control group (Table III; $P_{corr} < 0.009$). We did not find any differences in the frequency of the TNF308.2 allele between the two groups. The frequencies of TNF-promoter genotypes were in Hardy-Weinberg-equilibrium in both groups.

In a previous study on the association of HLA class I and class II alleles with chronic active hepatitis C, an increased frequency of DRB1*0301 and a decreased frequency of DRB1*1301/02 was found in the chronic hepatitis C group [Höhler et al., 1997], suggesting a predisposing and a protective effect, respectively. It was therefore of interest to determine whether any of these DRB1* alleles showed an association with certain TNF promoter alleles in the study subjects. There was a weak association between TNF238.2 and DRB1*0301 ($P < 0.003$; P_{corr} = n.s.), B18 ($P = 0.003$), and B57 ($P < 0.005$; P_{corr} = n.s.) in the chronic hepatitis C group but significance was lost after correction for the number of comparisons made (Table IV). The weak association of DRB1*0301 (DR17) and TNF238.2 was supported by analysis of eight DRB1*0301 homozygous patients in the chronic hepatitis C group, who were all heterozygous for the TNFA-A allele. Of the 27 haplotypes carrying a TNF238.2 alleles in the hepatitis C group only 8 were B18/DR3 or B57/DR7 positive. In an Italian study these haplotypes had been shown to be in strong linkage disequilibrium with the TNF238.2 allele [D'Alfonso and Richiardi, 1994]. Neither DRB1*1301/02 nor any of the other HLA-B or -DR antigens showed linkage disequilibrium to the TNF238.2 allele (Table IV).

DISCUSSION

A significant increase of the TNF238.2 promoter variant was found in patients with chronic active hepatitis C. There were significantly more heterozygous TNF238.2 individuals in the hepatitis C group. The study groups were too small to reach statistical significance for TNF238.2 homozygous individuals as these are very rare in the general population.

In a previous study on HLA class I and II associations in the same population, a significant increase of DRB1*0301 and a decreased frequency of DRB1*1301 and *1302 was found [Höhler et al., 1997] in the chronic hepatitis C group. The TNF238.2 variant has been shown to be in linkage disequilibrium with B18, B57, DR7, DR17.2, and to be part of the extended haplotypes B18-BFF1-DR17.2 (DRB1*0301) and B57-C4A6-DR7-DQw9 [D'Alfonso and Richiardi, 1994; Pociot et al., 1995]. Linkage disequilibrium to DR7 and only a weak one to DR3, B18, and B57 was found. Thirteen of 27 (48%) TNF238.2 variants occurred in individuals that were not DR3 positive. Although the observed association cannot be explained by linkage disequilibrium to the HLA-B or -DR genes a gene in the closer proximity of TNF-α, e.g., the complement genes C4, factor B, or C2 within the MHC class III region cannot be excluded. In a previous study on patients with HCV infection we did not find any significant association of these genes with chronic hepatitis C (unpublished data).

The TNF238.2 polymorphism lies in a putative regulatory box (Y-box) of the TNF-α promoter region which binds to regulatory DNA-binding proteins like NF-Y [D'Alfonso and Richiardi, 1994]. This putative regulatory box is believed to contribute to optimal promoter activity [Shakov et al., 1990]. Nucleotide position -238 is strongly conserved among different animal species raising the possibility that it is important functionally [Shakov et al., 1990]. A single base pair substitution in the Y-box of the HLA-DQA1 promoter causes decreased significantly transcription of the gene [Haas et al., 1995]. Similar observations have been reported for the human multidrug resistance gene [Goldsmith et al.,

TABLE III. Frequencies of TNF-α Alleles in the Chronic Hepatitis C Group Compared to Control Subjects*

	Controls (n = 198)		Chronic hepatitis C (n = 164)			
Allele	n	%	n	%	p	RR
TNF-α 238						
G	191	96.5	135	82.3	$p = 0.0001$; $p_{corr} < 0.009$	5.8
A	7	3.5	29	17.7		
TNF-α 308						
G	166	83.8	135	82.3	n.s.	
A	32	16.2	29	17.7		

*n = number of investigated chromosomes; n.s. = not significant.

TABLE IV. Linkage Disequilibrium Between HLA-DR and -B Alleles and the TNF238.2 Allele in Patients With Chronic Hepatitis C Virus Infection*

		TNF238.2 +	TNF238.2 −	N	p
B18	+	9	3	73	$p < 0.003$, p_{corr} = n.s.
	−	15	46		
B57	+	5	0	73	$p < 0.005$, p_{corr} = n.s.
	−	19	49		
DR3	+	14	11	73	$p < 0.003$, p_{corr} = n.s.
	−	10	38		
DR7	+	4	12	73	n.s.
	−	20	37		
DR13	+	0	6	73	n.s.
	−	24	43		

*Seventy-three patients were investigated for HLA-class I, II, and TNF promoter alleles. Linkage disequilibrium was tested using chi-square test.
n.s. = not significant.

1993] and the human thymidine kinase promoter [Lipson et al., 1989]. However, monocytes of TNF238.2 heterozygous subjects show no significant difference in TNF-α production after LPS treatment or allogenic stimulation [Pociot et al., 1995]. No significant difference was observed in the frequency of the TNF308.2 allele at position -308, which has been linked formerly to higher TNF-α production [Wilson et al., 1994] nor an increase of DRB1* alleles that have been linked to high TNF-α production [Jacob et al., 1990] (data not shown), it is considered that promoter activity changes caused by adenine at positon -238 are probably more complex than just a higher constitutive expression of the gene.

Viral infections can alter the expression of HLA and other accessory molecules that are important for immune recognition [Oldstone, 1989] and can also influence the transcription of cytokine genes. During hepatitis B virus infection HBcAg can suppress the transcription of interferon-β and the HBV polymerase protein has been shown to inhibit cellular responses to interferons α and γ [Foster et al., 1991; Whitten et al., 1991]. The infection of hepatocytes with either the hepatitis B or C virus induces the expression of TNF-α in these cells [González-Amaro et al., 1994].

The importance of cytokines like TNF-α and interferon-γ for the clearance of viral infections has been known for many years [Ramsay et al., 1993] and has only been emphasized recently by findings in a hepatitis B virus transgenic mouse model [Guidotti et al., 1995, 1996]. In this model it could be shown that only a minority of infected hepatocytes are eliminated by direct contact with cytotoxic T-cells whereas in the vast majority of infected cells HBV appears to be suppressed and eliminated by antigen-nonspecific cytokines [Guidotti et al., 1996]. Another recent study in patients with chronic hepatitis C showed that the presence of HCV RNA in the liver or in peripheral blood monocytes is associated with increased TNF-α expression [Larrea et al., 1996]. Furthermore, enhanced TNF-α synthesis was associated with nonresponsiveness to interferon-α treatment [Larrea et al., 1996]. Modifications of TNF-α expression by variations in the TNF-α promoter like the TNF238.2 allele might interfere with viral clearance and promote viral persistence. At present the influence of TNF-α on the course of HCV infection needs further investigation, which is hampered by the fact that there are no appropriate in vitro systems for HCV.

It was shown previously [Höhler et al., 1997] that immunogenetic factors are likely to influence the course of hepatitis C virus infection. The HLA-class II allele DRB1*1301-02 appears to have a protective effect against chronic infection whereas the TNF-α promoter allele 238.2 as well as DRB1*0301-02 [Höhler et al., 1997] are associated with a chronic disease course.

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REFERENCES

Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WS, Hu PYH, Miller JK, Gerber MA, Sampliner RE, Meeks EL, Beach MJ (1992): The natural history of community-acquired hepatitis C in the United States. New England Journal of Medicine 327:1899–1905.

Cabrera M, Shaw MA Sharples C, Williams H, Castes M, Convit J, Blackwell JM (1995): Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. Journal of Experimental Medicine 182:1259–1264.

D'Alfonso S, Richiardi PM (1994): A polymorphic variation in a putative regulation box of the TNFA promoter region. Immunogenetics 39:150–155.

Diepolder HM, Zachoval R, Hoffmann RM, Wierenga EA, Santantonio T, Jung MC, Eichenlaub D, Pape GR (1995): Possible mechanisms involving T-lymphocyte response to non-structural protein 3 in

viral clearance in acute hepatitis C virus infection. Lancet 346: 1006–1007.

Dyer P, Warrens A (1994): Design and interpretation of studies of the major histocompatibility complex in disease. In Lechler R (ed): "HLA and Disease." London: Academic Press, pp 93–121.

Foster GR, Ackrill AM, Goldin RD, Kerr IM, Thomas HC, Stark GR (1991): Expression of the terminal protein region of hepatitis B virus inhibits cellular responses to interferons alpha and gamma and double stranded RNA. Proceedings of the National Academy of Science 88:2888–2892.

Goldsmith ME, Madden MJ, Morrow CS, Cowan KH (1993): A Y-box consensus sequence is required for basal expression of the human multidrug resistance (mdr1) gene. Journal of Biological Chemistry 268:5856–5860.

González-Amaro R, García-Monzón C, García-Buey L, Moreno-Otero R, Alonso JL, Yagüe E, Pivel JP (1994): Induction of tumor necrosis factor α production by human hepatocytes in chronic viral hepatitis. Journal of Experimental Medicine 179:841–848.

Guidotti LG, Guilhot S, Chisari FV (1994): Interleukin-2 and alpha/beta interferon down-regulate hepatitis B virus gene expression in vivo by tumour necrosis factor-dependent and independent pathways. Journal of Virology 68:1265–1270.

Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV (1996): Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity 4:25–36.

Guo SW, Thompson EA (1992): Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Haas JP, Kimura A, Truckenbrodt H, Suschke J, Sasazuki T, Volgger A, Albert ED (1995): Early onset juvenile chronic arthritis is associated with a mutation in the Y-box of the HLA-DQA1 promoter. Tissue Antigens 45:317–321.

Hill AVS, Ruwende C, Mcuire W, Bellamy R, Coleman E, Ali S, Loke H, et al. (1996): Association of the TNF-238 promoter polymorphism with susceptibility to tuberculosis and malaria in Africa [abstract]. Human Immunology 47:118.

Höhler T, Gerken G, Notghi A, Knolle P, Lubjuhn R, Taheri H, Schneider PM, Meyer zum Büschenfelde KH, Rittner C (1997): MHC class II genes influence the susceptibility to chronic active hepatitis C. Journal of Hepatology 27:259–264.

Jakob CO, Fronek Z, Lewis G, Koo M, Hansen JA, McDevitt HO (1990): Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor α: relevance to genetic predisposition to systemic lupus erythematosus. Proceedings of the National Academy of Sciences USA 87: 1233–1237.

Koziel MJ, Dudley D, Afdhal N, Grakoui A, Rice CM, Choo QL, Houghton M, Ralston R, Walker BD (1995): HLA class-I-restricted cytotoxic T lymphocytes specific for hepatitis C virus. Identification of multiple epitopes and characterization of patterns of cytokine release. Journal of Clinical Investment 96:2311–2321.

Kuzushita N, Hayashi N, Katayama K, Hiramatsu N, Yasumaru M, Murata H, Shimizu Y, Yamazaki T, Fushimi H, Kotoh K, Kasaharah A, Fusamoto H, Kamada T (1996): Increased frequency of HLA DR13 in hepatitis C virus carriers with persistently normal ALT levels. Journal of Medical Virology 48:1–7.

Larrea E, Garcia N, Qian C, Civeira MP, Prieto J (1996): Tumor necrosis factor α gene expression and the response to interferon in chronic hepatitis C. Hepatology 23:210–217.

Lipson KE, Chen ST, Koniecki J, Ku DH, Baserga R (1989): S-phase-specific regulation by deletion mutants of the human thymidine kinase promoter. Proceedings of the National Academy of Sciences USA 86:6848–6852.

Löhr HF, Schlaak JF, Gerken G, Fleischer B, Dienes HP, Meyer zum Büschenfelde (1994): Phenotypical analysis and cytokine release of liver-infiltrating and peripheral blood T lymphocytes from patients with chronic hepatitis of different etiology. Liver 14:161–166.

McGuire W, Hill AVS, Allsopp CEM, Greenwood BM, Kwiatkowski D (1994): Variation in the TNF-α promoter region associated with susceptibility to cerebral malaria. Nature 371:508–511.

Missale G, Bertoni R, Lamonaca V, Valli A, Massari M, Mori C, Rumi MG, et al. (1996): Different clinical behaviours of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. Journal of Clinical Investment 98:706–714.

Oldstone MBA (1989): Viral persistence. Cell 56:517–520.

Pociot F, D'Alfonso S, Compasso S, Scorza R, Richiardi PM (1995): Functional analysis of a new polymorphism in the human TNF α gene promoter. Scandanavian Journal of Immunology 42:501–504.

Ramsay AJ, Ruby J, Ramshaw IA (1993). A case for cytokines as effector molecules in the resolution of virus infection. Immunology Today 14:155–157.

Shakov AN, Collart MA, Vassalli P, Nedospasov SA, Jongeneel CV (1990): κβ-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor a gene in primary macrophages. Journal of Experimental Medicine 171:35–47.

Tilg H, Wilmer A, Vogel W, Herold M, Nölchen B, Judmaier G, Huber C (1992): Serum levels of cytokines in chronic liver diseases. Gastroenterology 103:264–273.

Torre D, Zeroli C, Giola M, Ferrario G, Fiori GP, Bonetta G, Tambini R (1994): Serum levels of interleukin-1a, interleukin-1β, interleukin-6, and tumour necrosis factor in patients with acute viral hepatitis. Clinical Infectious Diseases 18:194–198.

Whitten TM, Quets AT, Schloemer RH (1991): Identification of the hepatitis B virus factor that inhibits expression of the beta interferon gene. Journal of Virology 65:4699–4704.

Wilson AG, De Vries N, Pociot F, Di Giovine FS, Van der Putte LBA, Duff GW (1993): An allelic polymorphism within the human tumor necrosis factor α promoter region is strongly associated with HLA A1, B8, and DR3 alleles. Journal of Experimental Medicine 177: 557–560.

Wilson AG, Symons JA, McDowell TL, Di Giovine FS, Duff GW (1994): Effects of a tumor necrosis factor (TNF-alpha) promoter base transition on transcriptional activity. British Journal of Rheumatology 33:89.

Zaykin D, Shivotovsky L, Weir BS (1995): Exact tests for associations between alleles at arbitrary numbers of loci. Genetica 96:169–178.